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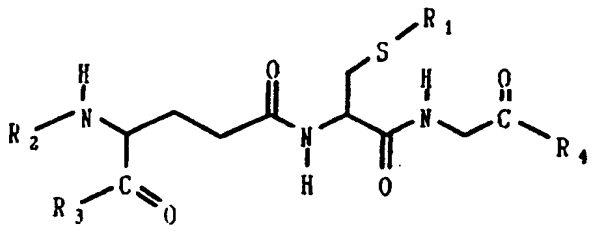
(54) Title: S-BLOCKED GLUTATHIONES



(I)

(57) Abstract

This invention concerns glutathione derivatives, and in particular their application in the suppression of pathogens. It has been discovered that certain glutathione derivatives are effective inhibitors of the growth of a range of cancer cell types, and certain micro-organisms. According to one aspect of the present invention there is provided a glutathione having structure (I). Compounds based upon this general structure are disclosed which are active against parasitic infectious agents such as T. Brucei and L. Donovani. Further compounds are disclosed which are active against cancer cells.

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S-Blocked Glutathiones

This invention concerns glutathione derivatives, and in particular their application in the suppression of pathogens.

Illness may be caused by many agents. Bacterial infections are caused by micro-organisms which multiply rapidly and cause a number of diseases. Parasites are organisms which live in or on a host and feed off the host. Cancers are evident by the uncontrolled multiplication of cells in the body. The cancer may be localized, such as breast cancer, or systemic such as leukaemia.

The treatment of the illnesses caused by the aforementioned agents has been the subject of much research, and many different approaches. One approach involves targeting the cells which cause the disease and destroying them or disrupting their ability to multiply.

For such an approach to be successful the agent or drug used to attack the diseased cells should not harm significantly other healthy cells. Thus such an

approach requires an understanding and identification of the biochemical processes carried out in cells, and the targeting and disruption of specific processes which are unique to the diseased cells.

Such an approach involves the use of the cytotoxic compound methylgloxal which is produced in the cells of certain organisms. A build up of methylgloxal in the cell up to cytotoxic levels will, of course, result in cell death. By inducing a build up of methylgloxal, significant growth inhibition effects have been seen in tumour cells, Escherichia coli, Saccharomycescerevisise and Leishmania donovani.

The build up of methylgloxal may be promoted by the inhibition of glyoxalase I (GLI) enzyme. A wide range of inhibitors of GLI are known and these include substrate or product analogues and mechanism-based inhibitors.

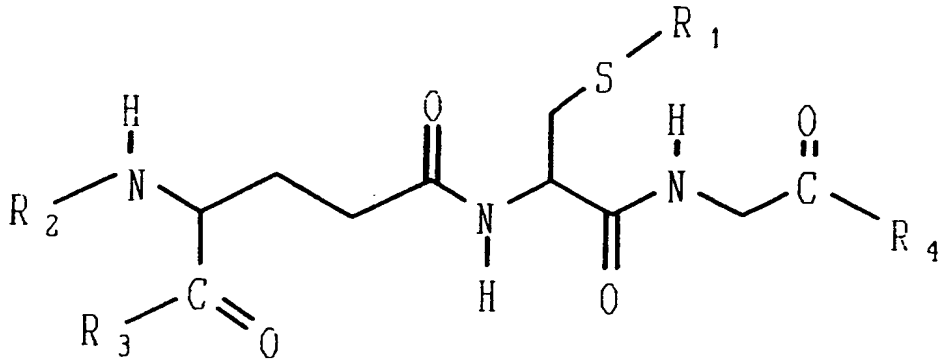
Glutathione (γ-glutamylcysteinylglycine) fulfils a variety of roles vital to life processes. It functions as a co-enzyme, co-substrate, substrate or part of the substrate architecture. S-blocked glutathiones have been shown to be potent inhibitors of GLI in vitro and this has led to a search for particular S-blocked

glutathiones which may have therapeutic effect.

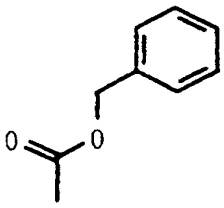
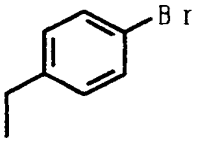
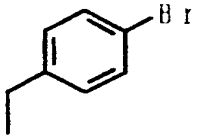
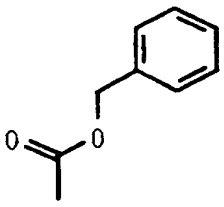
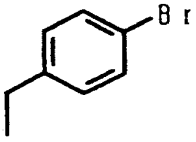
A general procedure for the preparation of the monoglycyl and dimethyl ester and amide derivatives of S-(4-bromobenzyl)glutathione has been described by the inventor in Biochem. J. (1990) 271, pp167-169.

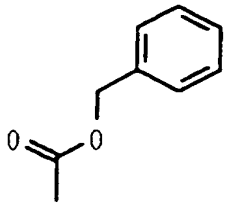
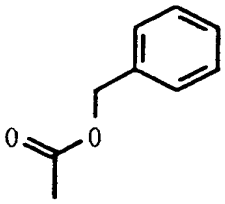
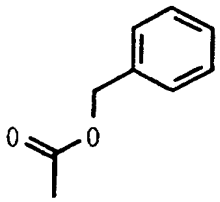
The inventor has discovered that certain glutathione derivatives are effective inhibitors of the growth of a range of cancer cell types, and certain micro-organisms.

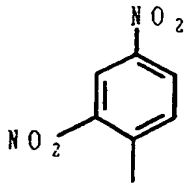
According to one aspect of the present invention there is provided a glutathione having the following structure:-

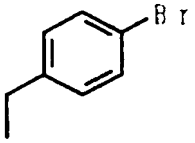
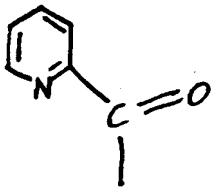
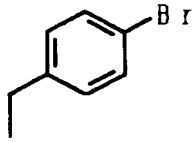
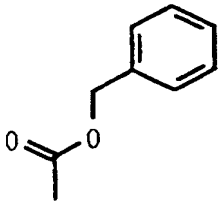
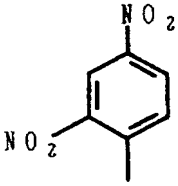
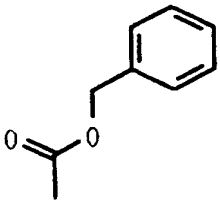
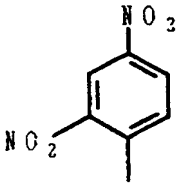


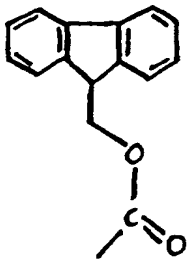
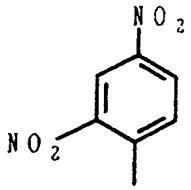
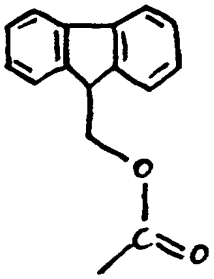
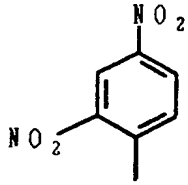
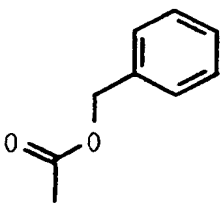
Where R1-R4 may be configured as shown in the following:

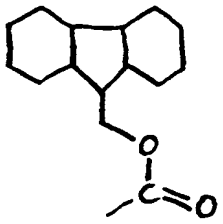
LABEL	R_2	R_1	R_4	R_3	MW
CD4			OMe	OMe	638.5
CD6	CH_3CO		OH	OH	545
CD7			OMe	OH	624

CD8		CH_2-COOEt	OH	OH	527
CD10			OMe	OMe	692

LABEL	R_2	R_1	R_4	R_3	MW
CD13	H		OH	OH	-

CD16	CHO		OH	OH	504
CD17			NH_2	OH	576
CD19			OMe	OH	621
CD20			OMe	OMe	635

CD42			OMe	OH	708
CD43			OMe	OMe	722
CD46		$-CH_2-CO_2Et$	OMe	OMe	555

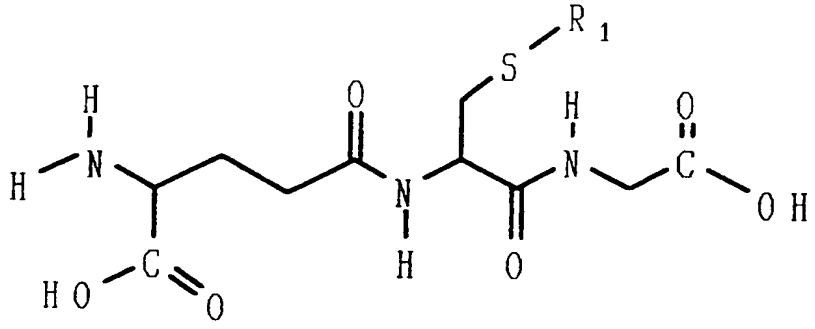
CD48	CH_3CO		OMe	OH	556

CD7 has been found to be an effective inhibitor of Trypanosomiasis and in particular T. Brucei S247. This compound is also active against Malaria. One particular advantage of this compound is that it is effective at inhibiting the cell growth of the organism without being toxic to red blood cells.

CD13 is effective at the inhibition of growth of cancer cells, and in particular leukaemia, breast cancer or tumour cells.

According to another aspect of the invention there is provided a glutathione having the following general formula:-

Wherein R1 is:-



-

$CH_2CH=C($

$CH_3)CH_2CH_2CH=C(CH_3)CH_2CH_2CH=C(CH_3)_2$

TRANS TRANS TRANS

S-(farnesyl)glutathione CD37

The aforementioned glutathione derivatives may each be provided in pharmaceutically acceptable compositions for delivery to the human or animal body.

In another aspect of the invention there is provided the use of any one of the foregoing compounds in the treatment of cancer, or parasitic cellular infestation.

In particular, according to one embodiment of the present invention there is provided the use of CD13 and derivatives thereof in the treatment of cancer.

According to another embodiment there is provided the

use of CD19 and/or CD20 in the treatment of a infection by a parasitic micro-organism.

In particular Cd19 is highly effective at low concentrations against T. brucei (African sleeping sickness), while CD 20 has no toxicity to red blood cells. CD20 also has good activity against other parasites such as L.donovani (oriental sore, Kala-azar).

According to another aspect of the invention there is provided the use of CD7 against malaria.

According to yet another aspect of the invention there is provided the use of any one of compounds CD26-CD37 against cancer.

According to another aspect of the invention there is provided the use of CD13, CD4, CD6 and CD 37 against cancer, and in particular breast cancer, and more particularly MCF7 cells.

Following is a description by way of example only of methods of putting the present invention into effect and examples demonstrating the activity of compounds according to the present invnetion. The drawing is a

graphic representation of the results of the in vivo test described in example 1.

General method of production.

$$GSH + RX \rightarrow GSR$$

Reduced glutathione (1g, 3.26mM) is dissolved in H_2O (5ml) and 2M NaOH (3.3ml, 6.6mM) with stirring at room temperature and under a nitrogen atmosphere. Ethanol (5-15ml) is then added to the cloud point whereafter RX (for example, aryl halide, 3.5mM dissolved in ethanol) is added portion-wise over about 30 minutes. The reaction is left to stir for 20 hours under nitrogen.

If precipitation occurs during addition either more ethanol or more water is added to dissolve the material. At the end the reaction the acidity of the mixture is adjusted to p 3.5 with 2M HCl and the mixture chilled to effect precipitation. The precipitate is then filtered, washed with water, dried and recrystallized from Ethanol/H_2O.

Pharmaceutical activity:

Example 1

Glutathione CD13 according to the present invention was tested in order to ascertain its inhibitory characteristics with respect to various cancer cell lines.

The compound was introduced to cell cultures and the concentration of cancer cells formed over a period of time was measured using standard techniques. The following table indicates the results for CD13 and another glutathione derivative "control" by way of comparison:-

	Leukaemia lines		Tumour cells	Breast Cancer cells
	WEH1 3B	K562	MAC 15A	MCF7
Compound	conc. µg/ml	conc. µg/ml	conc. µg/ml	conc. µg/ml
Control	36	54	>100	>100
CD13	3.4	2.5	0.43	0.48

CD13 was tested in vivo on rodents with MAC 15A S/C Tumours by giving them a 20 mg/Kg daily dose for five days. The treatment resulted in a reduction in tumour size and a 45% reduction in tumour volume after 4 days. The effect of CD 13 in the above test is shown

in the graph of the drawings.

Example 2

The glutathione derivatives CD7 and CD 10 according to the present invention were tested for their activity against the parasites T.Brucei S247, L.donovani and T. cruzi.

The following table shows the mean estimation of growth of T.Brucei S247 in a 72 hour incubation in the presence of a control glutathione derivative and CD10 in various concentrations:-

Compound	MIC @ concentration (μM)			
	30	10	3	1
control	++++	++++	+++++	+++++
CD7	0	++++	+++++	+++++
CD10	0	++++	+++++	+++++

The forgoing table shows that Compounds CD7 and CD10 exhibit complete inhibition of the growth of T.Brucei S247 over the specified period at concentrations of 30 μM.

The following table shows the results of the inhibition of the growth of L. donovani and T. Cruzi by compounds CD7, CD10 according to the present invention and "control" by way of comparison, at various concentrations.

Compound	% Inhibition of L.donovani @ conc.			% Inhibition of T. Cruzi @ conc.		
	90 (μM)	30 (μM)	10 (μM)	90 (μM)	30 (μM)	10 (μM)
control	0	0	0	0	0	0
CD7	0	0	0	0	0	0
CD10	T	T	0	T	T	T/0

The foregoing shows that compound CD10 shows good activity in the inhibition of growth of both L. donvani and T. cruzi.

Example 3:

Activity of compounds CD16-CD20 in Vitro.

Compound	% inhibition @ concn. (μ M)

	30	10	3	1
CD16				
T.cruzi	11.0	0	0	0
CD17				
T.cruzi	7.0	4.0	0	0
CD19				
T.brucei	100	100	33.2	0
CD20				
L.donovani	13.5	1.9	0	0
T.brucei	100	64.5	0	0

Example 4:

Compound CD48 was tested for its activity against cancer, with the following results for a range of cancer types:

Cancer	Human Ovarian carcinoma	Human lung carcinoma	Human colon carcinoma	Human myclogenious leukaemia	Mouse lymphoid neoplasm
Designation	A2780	H-460	BE	K562	P388

	µM	µM	µM	µM	µM
CD48	>50	>50	27.1	16.7	31.7

Example 5:

Compounds CD4, CD6, and CD 37 have also been found to be effective against various cancers, including breast cancer.

Example 6:

Compounds CD42 to 48 were tested for their activity against various parasitic infection agents and the results are shown in the following table:

Compound	% INHIBITION OF T.BRUCEI S247				%INHIBITION OF L. DONOVANI			
	30µM	10µM	3µM	1µM	30µM	10µM	3µM	1µM
CD42	100	100	0	0	1.2	0	0	0
CD43	100	100	0	0	T/O	0	0	0
CD44	100	100	100	63.2	T/ 100	0	0	0

CD46	0	0	0	0	66.2 3	0	0	0

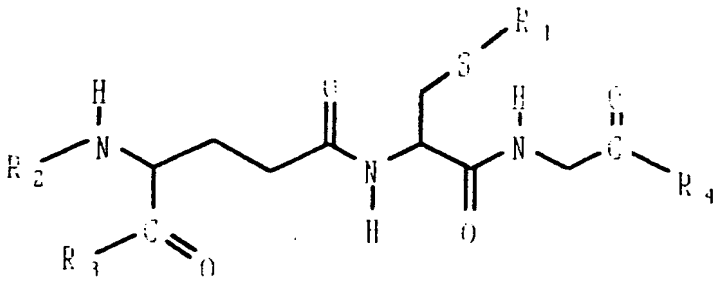
Key: T/0 = toxic to macrophages / parasites present.

T/100 = toxic to macrophages / no parasites present.

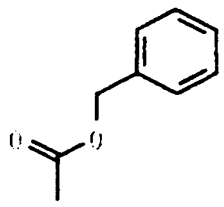
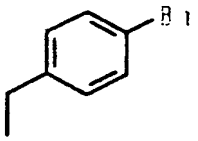
100+ = 100% inhibition when sampling for haemocytometer count but parasites visible in wells of 96-well plate under inverted mic.

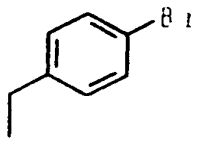
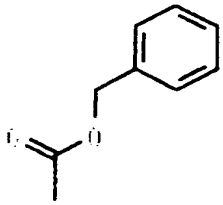
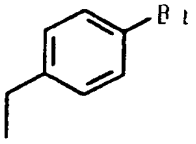
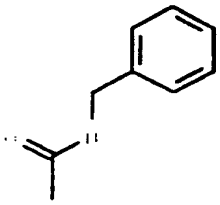
CLAIMS

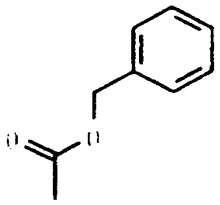
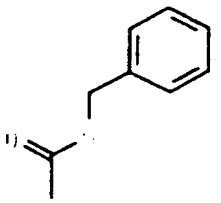
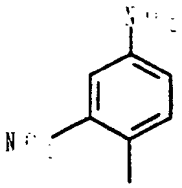
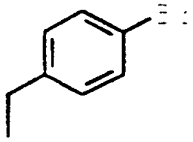
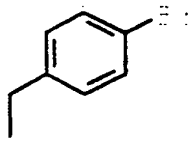
1. A compound having the following general structure:-

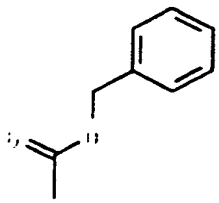
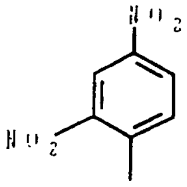
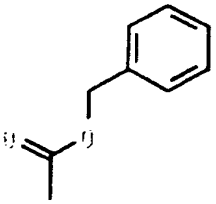
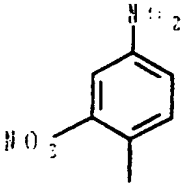


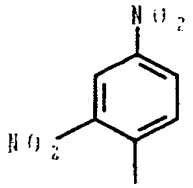
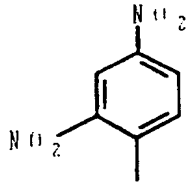
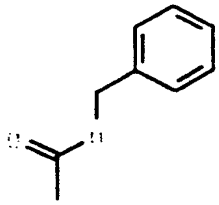
and wherein R_1 to R_4 are according to any one of the following rows in the table:

LABEL	R_2	R_1	R_4	R_3
CD4			OMe	OMe

CD6	CH_3CO		OH	OH
CD7			OMe	OH
CD8		CH_2-COOEt	OH	OH

CD10			OMe	OMe
CD13	H		OH	OH
CD16	CHO		OH	OH
CD17			NH_2	OH

CD19			OMe	OH
CD20			OMe	OMe
CD37	H	$\neg CH_2CH=C(CH_3)CH_2CH_2CH=C(CH_3)CH_2CH_2CH=C(CH_3)_2$	OH	OH

CD42			OMe	OH
CD43			OMe	OMe
CD46		$-CH_2-CO_2Et$	OMe	OMe

CD48	CH_3CO		OMe	OH

2. A pharmaceutically acceptable composition for delivery to the human or animal body comprising a compound according to claim 1.

3. A composition for the treatment of parasitic infection, which composition comprises one or more of CD7, CD19, CD20, CD42, CD43 or CD44 according to claim 1.

4. A composition for the treatment of trypanosomiasis, and in particular infection by T. Brucei, which composition comprises one or more of CD7, CD19, CD20, CD42, CD43 and CD44 according to claim 1.

5. A composition as for the treatment of Leishmaniasis, and in particular infection by L. donovani, which composition comprises one or more of CD20, CD42, CD43, CD44 and CD46 according

to claim 1.

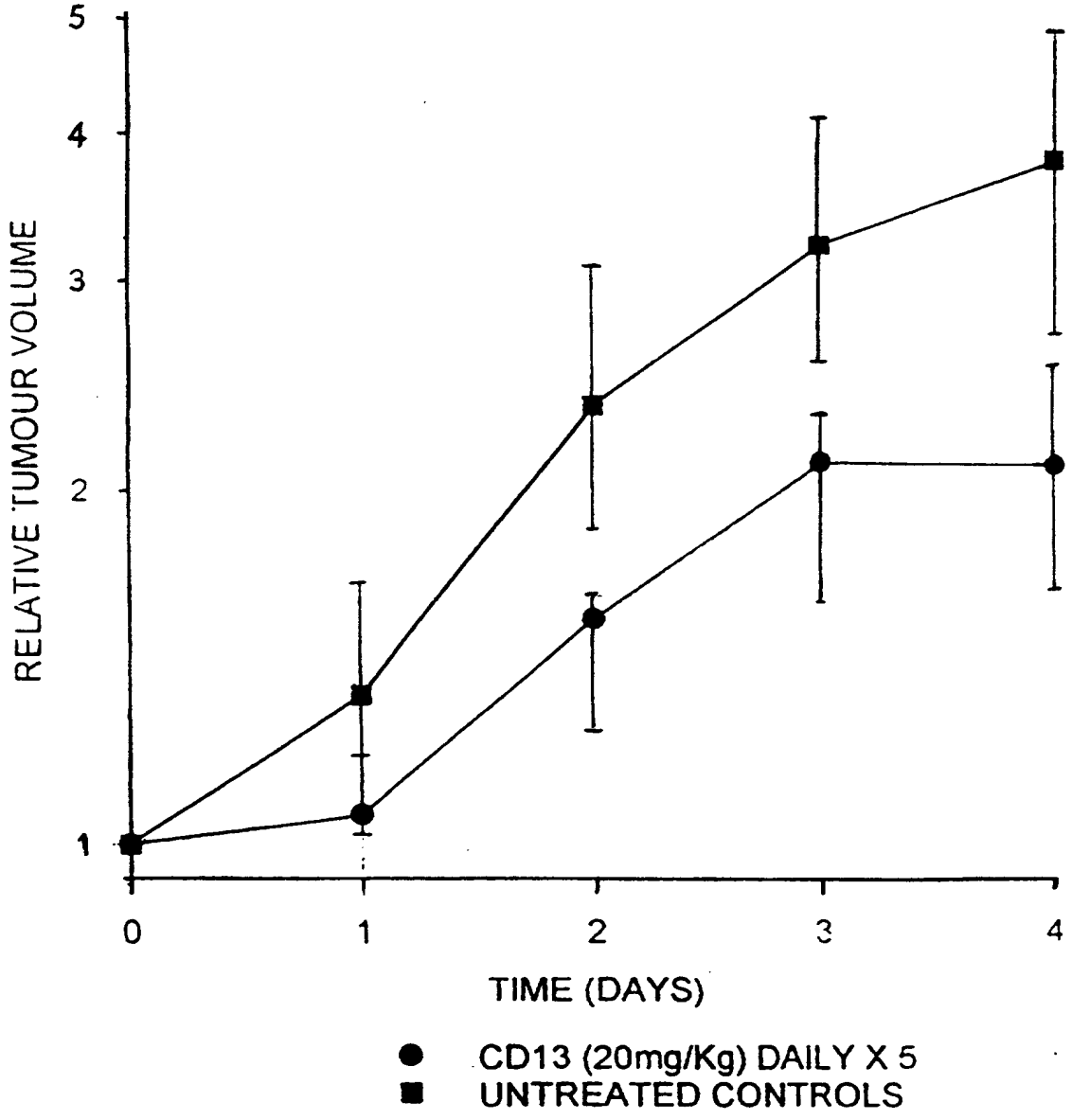
6. A composition for the treatment of cancer, which composition comprises one or more of CD4, CD6, CD13 and CD37 according to claim 1.

7. A method of treating a diseased human or animal comprising administering a pharmologically effective amount of a composition as claimed in claim 2.

8. A method of treating parasitic infection of a human or animal comprising administering a pharmologically effective amount of a composition as claimed in any of claims 3,4 and 5.

9. A method of treating cancer in a human or animal comprising administering a pharmologically effective amount of a composition as claimed in claim 6.

MAC15A S/C TUMOURS TREATED WITH CD13 DAILY



INTERNATIONAL SEARCH REPORT

Intern. nal Application No
PCT/GB 97/02358

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K5/02 A61K38/05

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	R.VINC ET AL.: "Studies on the Inhibition of Glyoxalase I by S-Substituted Gluthationes" J.MED.CHEM., vol. 14, no. 5, 1971, page 402-4 XP002044716 see paragraph 1; example 39; table 1 ---	1,6-9
X	A.AL-TIMARI ET AL.: "Inhibition of mammalian glyoxalase I (lactoylglutathione lyase) by N-acylated S-blocked glutathione derivatives as a probe for role of the N-site of gluthathione in glyoxalase I mechanism" BIOCHIM.BIOPHYS.ACTA, vol. 86, no. 1, 1986, pages 160-8, XP002044717 see tables 12,5 ---	1,6-9

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
27 October 1997	13. 11. 97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel: (+31-70) 340-2040; Tx: 31 651 epo nl; Fax: (+31-70) 340-3016	Deffner, C-A

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INTERNATIONAL SEARCH REPORT

Intern. nal Application No
PCT/GB 97/02358

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	A.AL-TIMARI ET AL.: "Inhibition of glutathione derivatives of bovine liver glyoxalase II (Hydroxyacylgluthatione hydrolase) as a probe of the N- and S-sites for substrate binding" BIOCHIM.BIOPHYS.ACTA, vol. 870, no. 2, 1986, page 219-25 XP002044718 see example 2; table I	1,6-9
A	S.J.NORTON ET AL.: "Inhibitors and inhibition of mammmalian glyoxalase II activity" BIOCHEM.SOC.TRNS., vol. 21, no. 2, 1993, pages 545-9, XP002044719	
A	NORTON S.J. ET AL.: "Glyoxalase and Glyoxalase II from Aloe Vera: Purification, Characterization and Comparision with Animal Glyoxalases" BIOCHEM.INT, vol. 22, no. 3, 1990, pages 411-18, XP002044720	
A	AC. ELIA ET AL.: "N,S-Bis-Fluorenylmethoxycarconylglutathione: A New, Very Potent Inhibitor of Mammalian Glyoxalase II" BIOCHIM.MOL.BIOL.INT., vol. 35, no. 4, 1995, pages 763-71, XP002044721	
A	WO 95 08563 A (TERRAPIN TECH INC) 30 March 1995	

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. nal Application No
PCT/GB 97/02358

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9508563 A	30-03-95	US 5599903 A	04-02-97
		AU 7842194 A	10-04-95
		CA 2171453 A	30-03-95
		EP 0720620 A	10-07-96
		JP 9506336 T	24-06-97
		US 5556942 A	17-09-96